REPORT DOCUMENTATION PAGE

Form Approved
OBM No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 8-10	Proceedings

4. TITLE AND SUBTITLE

The Role of Oceanospirillum Exopolymer In Marine Copper Corrosion

5. FUNDING NUMBERS

Job Order No. 573505204
Program Element No. 0601153N
Project No. 03103
Task No. 320
Accession No. DN094463

6. AUTHOR(S)

[1]P. Angell, [1]A. Sonnerson, [1]D. C. White, P. Wagner, and B. Little

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
Oceanography Division
Stennis Space Center, MS 39529-5004

8. PERFORMING ORGANIZATION REPORT NUMBER

NRL/PP/7333--95-0046

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
Oceanography Division
Stennis Space Center, MS 39529-5004

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

1995 International Conference on Microbially Influenced Corrosion, May 8-10, 1995, New Orleans, LA
[1]Center for Environmental Biotechnology, 10515 Research Dr., Suite 300, Knoxville, TN 37932

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The marine bacterium *Oceanospirillum* produces copious amounts of exopolymer when grown on copper surfaces and has been shown to be involved in the corrosion of 99% copper. A study was undertaken to determine the nature of the exopolymer and its possible role in corrosion processes. *Oceanospirillum* was grown in small laminar flow cells with copper and 316 stainless steel as substrata. Exopolymer was harvested, purified, quantified and chemically characterized. Copper promoted greater polymer production than stainless steel. Exopolymers from both substrata contained glucose with no other sugar monomers or uronic acids.

14. SUBJECT TERMS

biofouling; corrosion; biodeterioration; electrochemistry

15. NUMBER OF PAGES

9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

1995 INTERNATIONAL CONFERENCE ON MICROBIALLY INFLUENCED CORROSION

May 8–10, 1995
New Orleans, Louisiana

Sponsored and Organized by:

American Welding Society

NACE INTERNATIONAL
Informing the World on Corrosion Control

Editors:
P. Angell
S.W. Borenstein
R.A. Buchanan
S.C. Dexter
N.J.E. Dowling
B.J. Little
C.D. Lundin
M.B. McNeil
D.H. Pope
R.E. Tatnall
D.C. White
H.G. Ziegenfuss

TABLE OF CONTENTS

The Role of *Oceanospirillum* Exopolymer In Marine Copper Corrosion

Peter Angell
Center for Environmental Biotechnology
10515 Research Drive, Suite 300
Knoxville, TN 37932

Anders Sonnerson
Center for Environmental Biotechnology
10515 Research Drive, Suite 300
Knoxville, TN 37932

Patricia Wagner
Naval Research Laboratory
Stennis Space Center, MS 39529

David C. White
Center for Environmental Biotechnology/Oakridge National Laboratory
10515 Research Drive, Suite 300
Knoxville, TN 37932

Brenda Little
Naval Research Laboratory
Stennis Space Center, MS 39529

Abstract

The marine bacterium *Oceanospirillum* produces copious amounts of exopolymer when grown on copper surfaces and has been shown to be involved in the corrosion of 99% copper. A study was undertaken to determine the nature of the exopolymer and its possible role in corrosion processes. *Oceanospirillum* was grown in small laminar flow cells with copper and 316 stainless steel as substrata. Exopolymer was harvested, purified, quantified and chemically characterized. Copper promoted greater polymer production than stainless steel. Exopolymers from both substrata contained glucose with no other sugar monomers or uronic acids.

Oceanospirillum produced local anodic regions on copper surfaces as detected with scanning vibrating electrode microscopy (SVEM). Anodic regions corresponded to areas with high numbers of bacteria.

Keywords: copper, *Oceanospirillum*, SVEM, exopolymer

Introduction

Copper and copper alloys have long been successfully used in marine applications because of good mechanical strength and workability, corrosion resistance, electrical and thermal conductivity and resistance to macrofouling. Applications include distribution and piping systems, heat exchangers, intake screens and sheathing for splash zones, as well as offshore structures.[1–3] The corrosion product cuprous oxide, cuprite (Cu_2O), forms on all copper surfaces in oxygenated seawater.[4] Copper ions and electrons pass through this film, dissolve in the water and precipitate as $Cu_2(OH)_3Cl$ independent of alloy composition.[5] The film retards anodic dissolution and the rate of oxygen reduction. Corrosion failures in copper alloys are associated with the breakdown of $Cu_2(OH)_3Cl$ by mechanical[6], chemical[7], or biological means.[8]

Biofilms develop on all surfaces exposed to natural marine environments. Cuprous oxide is commonly used in marine antifouling coatings to retard macrofouling. However, bacteria, microalgae, protozoa and their exudates readily form slime layers on copper-containing surfaces.[9] Although Leifson et al.[10] described "large spirilla with characteristic curved soma and lophotrichous flagella" as rare in seawater, *Oceanospirillum* has been invariably isolated from U. S. Navy platforms in locations throughout the world. The platforms had in common that they were all coated with copper-containing antifouling coatings. *Oceanospirillum* (Figure 1) is an aerobic, heterotrophic, motile, helical bacterium with rigid cell walls and requires sodium chloride for growth.[11] In experiments conducted by A. V. Stiffey, it was demonstrated that *Oceanospirillum* not only colonized copper-containing surfaces, but also detoxified copper ions from the substrata so that non-copper tolerant bacteria settled on surfaces previously colonized with *Oceanospirillum*.[12] Wagner et al.[13] demonstrated that the organism produces copious amounts of exopolymer when grown on copper and that the organism and associated polymers bind copper ions from solution or substrata. They used electrochemical techniques to demonstrate that the organism increased the corrosion rate of 99% copper in a seawater medium.

Daniel et al.,[14] Chamberlain et al.[15–16] and Geesey et al.[17] stressed the relationship between microbial exopolymers and copper corrosion in fresh water systems. Each group emphasized the role of exopolymers in binding copper and the formation of copper concentration cells. The experiments described in this paper were designed to determine the nature of the *Oceanospirillum* exopolymer and its possible role in copper corrosion processes in marine waters.

Materials and Methods

Bacterium and Medium

Oceanospirillum was isolated at the Naval Research Laboratory, Stennis Space Center, MS, and maintained by weekly transfers on marine agar and incubation at 25°C. Liquid medium was used for all experiments and contained the following (g/l): sea salts 40, glutamic acid 1.81, ammonium chloride 0.0064, sodium phosphate monobasic 0.00138. Flow cells filled with 10 ml of medium were inoculated and cells grown overnight.

Exopolymer Characterization

Exopolymer for characterization was generated by growing *Oceanospirillum* on 99% copper or 316 stainless steel coupons in laminar flow cells described previously by Mittelman et al.[18] Biofilms were allowed to grow for 168 hrs before being harvested by sonication. Biofilms were resuspended in sterile seawater and treated with 0.8 ml of 5 M sodium chloride and 0.5 M EDTA to aid removal of exopolymer from bacteria. Bacterial cells were then removed by centrifugation. The supernatant was treated with 3 volumes of propan-1-ol at 4°C to precipitate exopolymer which was then dialyzed against running tap water overnight followed by dialysis against 18 MΩ water. Purified exopolymer was then lyophilized and stored at 4°C. Monomer characterization was carried out as described previously.[19] In summary, exopolymer was hydrolyzed using 4M trifluroacetic acid at 100°C for 2 hours. Lipids were removed by passing through a C_{18} column before high pressure anion exchange chromatography separations of 25 µl samples were performed with a pellicular CarboPac PA1 anion exchange column on a Dionex(1) series 4500 high-pressure liquid chromatography system. X-ray absorption near edge structure (XANES) was used to determine the speciation of copper within exopolymers.[20]

---

(1) Dionex Corp., Sunnyvale, CA 94088

Scanning Vibrating Electrode Microscopy (SVEM) Studies

Coupons colonized by *Oceanospirillum* and sterile medium controls were examined with the SVEM system.[21] The SVEM system consisted of a 20 μm stainless steel microprobe electroplated with platinum black that is vibrated in two orthogonal directions while scanned under computer control over a coupon surface. The probe is capable of capacitively measuring local current density at each point of the scan, allowing the computer to generate maps of the local current densities over a surface. The vibrating probe system is mounted under a microscope fitted with reflected light Nomarski and epifluorescence systems (40× water objective, 2 mm working distance) and an imaging system. Current density scans can be concurrently mapped with images of the surface.

Results and Discussion

Biofilm Generation and Characterization

Biofilms formed readily on both copper and stainless steel coupons. The color of extracted biofilm was variable, particularly in the case of the copper. When the medium was recycled through the system containing copper coupons, it turned blue, presumably due to copper ions picked up by the glutamic acid. Extracted biofilms were also blue, especially in areas where air had become trapped in air pockets over parts of the coupon surface.

Following extraction and purification, exopolymer from each system was weighed. Exopolymer recovered from copper and stainless steel coupons weighed 9.6 mg and 4.3 mg, respectively. The recoveries corresponded to 0.74 mg cm^{-2} and 0.33 mg cm^{-2} copper and stainless steel, respectively, indicating that roughly twice as much polymer was generated under similar conditions on copper compared to stainless steel.

Polymer from both surfaces consisted of glucose units with no other monomers. No uronic acid residues were detected in any of the exopolymers. XANES spectra of exopolymers indicated the presence of bound Cu^{+2}. Additional work is underway to interpret binding site symmetry and the nature of the chemical surroundings.

SVEM Observations

Sterile Control. SVEM studies of sterile copper controls showed no localized anodic activity after 200 hrs when medium levels were maintained. Under normal operating conditions no coloration of the medium in SVEM experiments was noted unless fluid levels were reduced, forming a thin film of liquid over the copper coupons, in which case a heavy blue coloration was noted. Occasionally a thin gel formed. Scans conducted immediately after the liquid level was restored showed strong anodic activity which persisted for up to 18 hours.

Oceanospirillum Culture. When copper coupons were inoculated with *Oceanospirillum* several localized anodic regions were observed after about 48 hrs Anodic regions persisted until termination of the experiment at 180 hrs. Anodic currents were always present but intensities varied. One anodic region was stronger and more persistent than others (Figure 2). That anodic region correlated with a dark area detected microscopically (Figures 3 and 4).

At the conclusion of the experiment, coupons were stained with a fluorescent stain (Live/Dead Backlight Viability Kit(2)) that distinguishes live bacterial cells. All bacteria on the surface were alive. Scanning the sample using the 40× water immersion lens demonstrated that bacterial colonization was not uniform.

(2) Molecular Probes, Eugene, OR

Instead, micro- and macro-colonies had formed. Areas with higher densities of bacteria correlated to anodic regions. The dark area in Figure 3 contained high numbers of bacteria and sustained local anodic activity.

Conclusions

The marine bacterium *Oceanospirillum* (1) produces copious amounts of exopolymer when grown on copper surfaces (2) binds Cu^{+2} from the substratum and (3) produces local anodic regions on copper surfaces detected with SVEM. The exopolymer contained glucose with no other sugar monomers or uronic acids.

Acknowledgments

This work was performed under Program Element 0601153N, NRL Contribution number NRL/PP/733--95-0046. The authors are grateful for assistance from E. Karplus (Sciencewares, E. Falmouth, MA) and K. Klutz (Georgia Instruments, Roswell, GA) in setting up the SVEM.

References

1. G. Blunn, in Biodeterioration VI (Slough, U.K.: CAB International, 1987), p. 567.

2. B. B. Moreton and T. J. Glover, "New Marine Industry Applications for Corrosion and Biofouling Resistant Copper-Nickel Alloys," 5th International Congress on Marine Corrosion and Fouling, (Barcelona, Spain: Biologia Marina, 1980), p. 267.

3. W. W. Kirk, Materials Performance, 23 9 (1987).

4. R. F. North and M. J. Pryor, Corrosion Science, 10 (1970): p. 297.

5. S. R. deSanchez and D. J. Schiffrin, Corrosion Science, 22 (1982): p. 585.

6. S. Sato and K. Nagata, Light Metal Technical Reports, 19 3 and 4 (1978): p. 83.

7. B. C. Syrett. D. D. Macdonald and S. S. Wing, Corrosion, 35 9 (1979): p. 409.

8. E. C. Fischer, V. J. Castelli, S. D. Rodgers and H. R. Bleile, in Marine Biodeterioration: An Interdisciplinary Study, J. D. Costlow and R. C. Tipper, eds., (Annapolis, MD: Naval Institute Press, 1981), p. 261.

9. S. M. Gerchakov and L. R. Udey, in Marine Biodeterioration: An Interdisciplinary Study, J. D. Costlow and R. C. Tipper, eds., (Annapolis, MD: Naval Institute Press, 1981), p. 82.

10. E. Leifson, B. J. Cosenza, R. Murchelano and R. C. Cleverdon, J. Bacteriology, 87 3 (1964): p. 652.

11. Bergey's Manual of Determinative Bacteriology, R. E. Buchanan and N. E. Gibbons, eds., (Baltimore, MD: Wilkins and Wilkins Co., 1975), p. 72.

12. A. V. Stiffey, personal communication, 1990.

13. P. A. Wagner, B. J. Little and A. V. Stiffey, An Electrochemical Evaluation of Copper Colonized by a Copper-Tolerant Marine Bacterium, CORROSION/91, paper no. 109, (Houston, TX: National Association of Corrosion Engineers International, 1991).

14. G. F. Daniel, A. H. L. Chamberlain and E. B. G. Jones, Helgolander Meeresunters, 34 (1980): p. 123.

15. A. H. L. Chamberlain, P. Angell and H. S. Campbell, British Corrosion J., 23 (1988): p. 197.

16. A. H. L. Chamberlain and P. Angell, Influences of Microorganisms on Pitting of Copper Tube, in: Microbially Influenced Corrosion and Biodeterioration, N. J. Dowling, M. W. Mittleman and J. C. Danko, eds., (Knoxville, TN: Univ. of Tennessee, 1991), p. 3–65.

17. G. G. Geesey, M. W. Mittelman, T. Iwaoka and P. R. Griffiths, Materials Performance, 25 2 (1986): p. 37.

18. M. W. Mittelman, L. L. Kohring, and D. C. White, Biofouling, 6 (1992): p. 39–51.

19. M. J. Franklin, C. E. Chitnis, P. Gacesa, A. Sonesson, D. C. White and D.E. Ohman, Journal of Bacteriology, 176 (1994): p. 1821–1830.

20. A. Bianconi and A. Marcelli: Synchrotron Radiation Research: Advances in Surface and Interface Science, Techniques, R. Z. Bachrach, ed., (New York: NY, Plenum Press, 1992), p. 63.

21. P. Angell, J-S. Luo and D. C. White, Use of 2-D Vibrating Electrode Technique in MIC Studies, CORROSION/94, paper no. 266, (Houston, TX: National Association of Corrosion Engineers International, 1994).

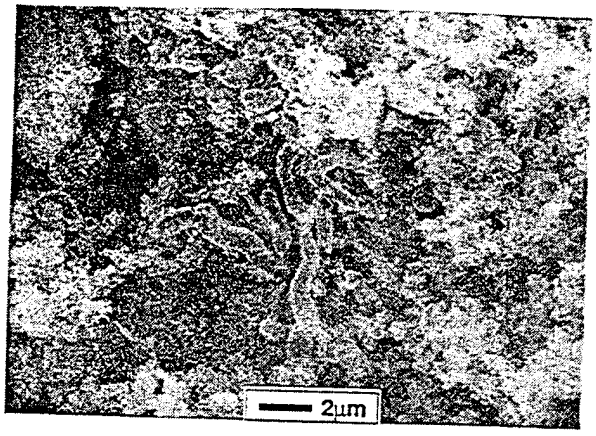

Figure 1. *Oceanospirillum* grown on copper surface.

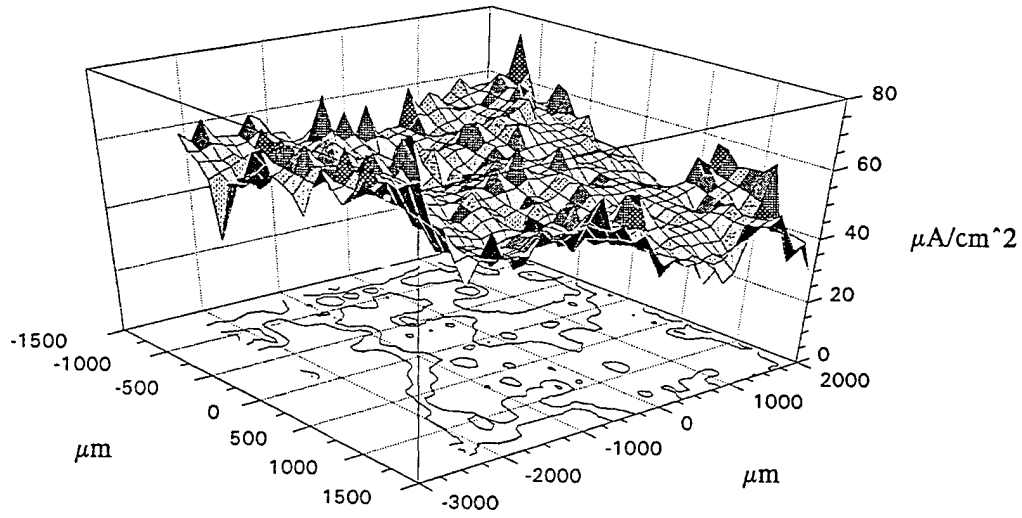

Figure 2. Current density scan over a copper coupon exposed to *Oceanospirillum* for 160 hours showing several anodic regions with a large area visible to the back left corner.

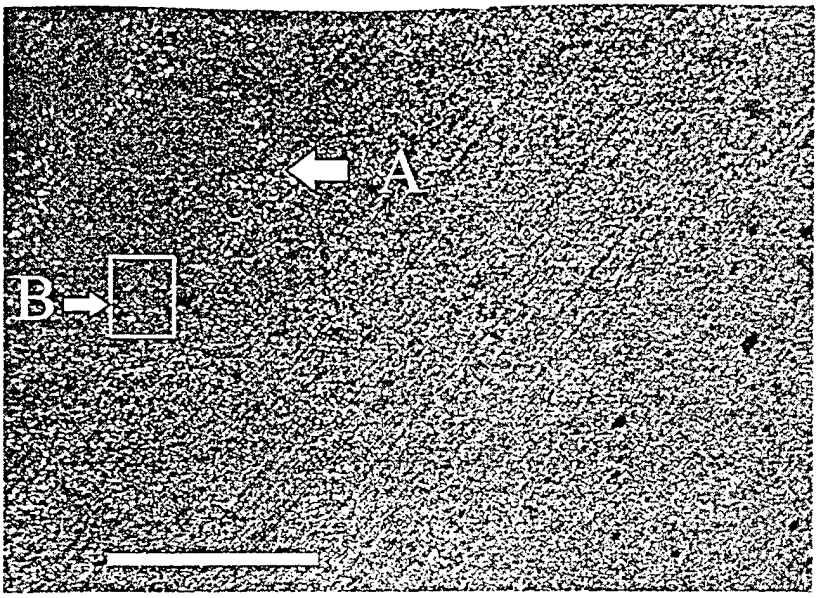

Figure 3. Low power image of copper coupon after 160-hour exposure to *Oceanospirillum*, showing an area of darkening (A) corresponding to the area of anodic current seen in Figure 2. The boxed are towards the bottom of the darkened area (B) is shown at higher power in Figure 4 (bar = 1 mm).

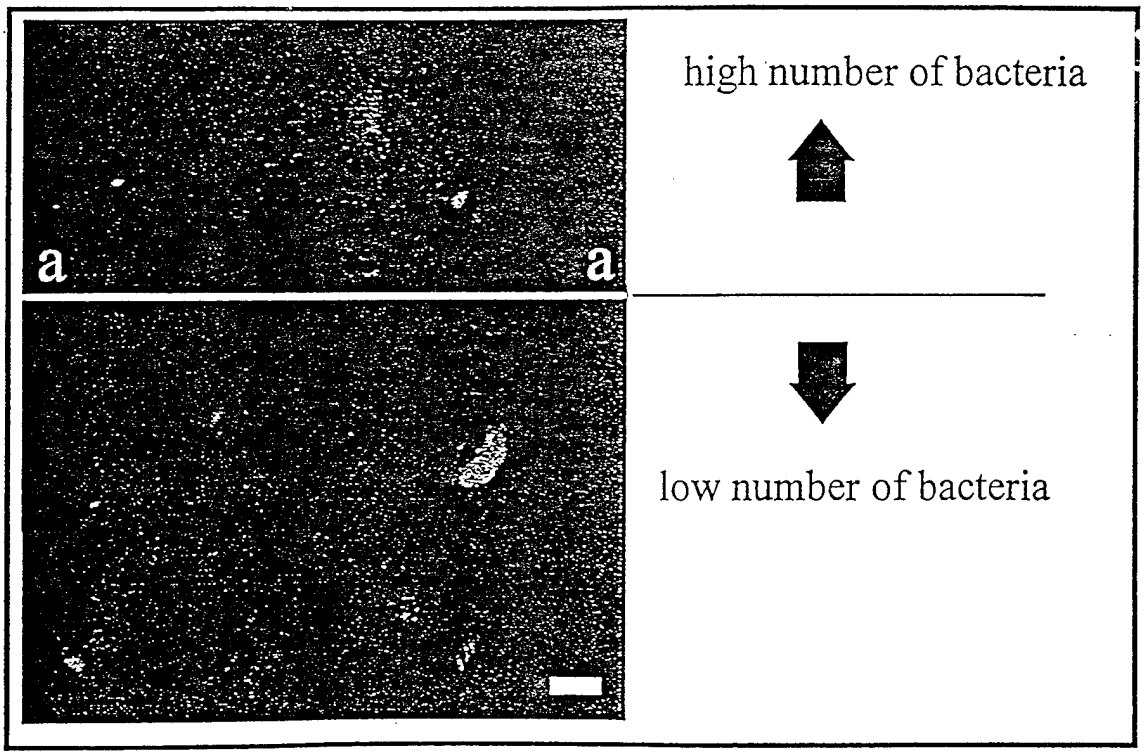

Figure 4. Fluorescent image showing bacteria (small white dots) in highlighted area in Figure 3, showing high number of bacteria above line (dark area in Figure 3) and low number of bacteria below line (bar = 20 μm).